Journal of Aging Research & Clinical Practice©
Volume 2, Number 4, 2013

SELENIUM STATUS IN A 12 MONTH LONGITUDINAL STUDY OF OLDER TASMANIAN ADULTS

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Abstract: *Background:* Selenium is important in many areas of health including immune and antioxidant function. Inadequate selenium status in the elderly is common, and may be particularly important as immune function decreases and risk of chronic and other disease increases. Little data has been reported about medium term variation in selenium status. *Objectives:* To investigate the magnitude of variation in selenium status of older adults over 12 months and the influence of season. *Design:* A 12 month longitudinal study. *Setting:* Independent, community dwelling self-selected older adults in 2009 and 2010. *Participants:* 80 adults (23 men, 57 women) in Australia, aged 60 years or older. *Measurements:* Dietary selenium intake was assessed using a food frequency questionnaire. Serum selenium concentration was measured using graphite furnace atomic absorption spectroscopy. *Results:* At commencement, men consumed 80.6 μg, and women 66.8 μg selenium per day, respectively; there was however, no significant difference in serum selenium (1.11 v. 1.09 μmol/l; P = 0.58). Repeated measures nonlinear regression analysis revealed the mean magnitude of variation over 12 months was small and non-significant (0.02 μmol/l; 95% CI -0.01 to 0.05; P = 0.17). Overall there was minimal variation over the study period; greatest variation was observed in subjects in the upper quartile of selenium status at commencement; the mean SD of serum selenium change compared to all others was 0.15 vs. 0.07 μmol/l (95% CI of difference 0.04 to 0.13; P<0.001). *Conclusions:* In these older adults, selenium status did not vary significantly over 12 months and there was no evidence of a seasonal pattern.

Key words: Selenium, older adults, longitudinal studies, Australia.

Introduction

As an essential component of some 25 proteins, selenium has a significant influence on human health (1). Keshan disease results from overt selenium deficiency, while milder inadequacies may be of importance in many more people worldwide. Research has elucidated a variety of roles for selenium such as those in antioxidant protection, the immune system, thyroid function, neurological function, and reproduction (2). Much effort has also been expended in examining the relationship between low selenium status and a number of chronic conditions (3), in particular, cancer (4).

Known for its wide regional variations, selenium status is also often reported to decline in old age (5-7). Several studies have shown inverse relationships between selenium status and mortality and morbidity in older age groups (8-10). However, recent studies have also reported associations between higher selenium status and hypertension (11), diabetes (12) and increased lipid levels (13).

Given the role of selenium in immune and antioxidant function, it is likely to be important for the maintenance of health during the aging process. Thus in many populations with sub-optimal selenium status, the older age groups may be at greatest risk of potential adverse health effects resulting from low selenium status, which could be exacerbated should seasonal changes occur.

Previously, there has been some suggestion of seasonal differences in mean (plasma/serum) selenium concentrations in cross-sectional studies (5, 14); however, to date the variability of selenium status of individuals over the medium term (≈ 12 months) has not been investigated, nor has the potential for seasonal differences.

In Tasmania, the southernmost state of Australia, and a region with low soil selenium levels in major agricultural areas, marginal selenium status appears common. In our recent population study of selenium status in Tasmania (15), and in line with reports from elsewhere of declining selenium status with age (5, 6), the oldest subjects (75-84 yrs) had the lowest mean serum Se concentrations; 1.03 μmol/l compared to the overall sample mean of 1.13

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Received June 17, 2013
Accepted for publication August 18, 2013

μmol/l. As has been noted in the literature, selenium content of foods appears to vary widely in Australia (16); and, as well as wide regional differences, seasonality has been observed in important dietary sources of selenium such as dairy (17). In the current study we hypothesize that there may be seasonal variation in selenium status in the human population of Tasmania due to seasonal changes in food content as well as the types of food eaten. As older members of the Tasmanian population appear at greater risk of low selenium status, such variation could be of particular significance. Therefore our aim was to assess the selenium status of people aged 60 years and above, over 12 months, to determine the magnitude of any variation over this time and whether a seasonal pattern could be detected.

Methods

This was a 12 month longitudinal observational study conducted between 2009 and 2010. It was part of a larger study investigating vitamin D status and its association with sun exposure, balance and muscle strength in 91 self-selected older adults, data from which has been reported previously (18, 19). Recruitment of community-dwelling adults over the age of 60 years was via advertisements in local newspapers and through community groups. The sample for this study consisted of 23 men and 57 women free from significant illness and residing in north, north-west and southern Tasmania, in both rural and suburban areas. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Research Ethics Committee (Tasmania). Written informed consent was obtained from all subjects.

The study collected data at three-monthly intervals over 12 months, resulting in five separate time points; the last data collection occurring in the same month as the first, in the following year, for each subject. As well as time point comparisons, the study was designed to analyse the 12 month variation in serum selenium status using a sine wave model. As we hypothesized that seasonal changes in dietary factors could lead to a seasonal variation in serum selenium, the sine wave pattern of solar UVB radiation levels was used as a proxy for season (18). As a trial of the study procedures, the commencement of the study was staged such that a portion of participants (n = 16) began their 12 month involvement three months prior to the rest of the cohort.

At each time point anthropometric and dietary data, and information on supplement use was collected. Dietary intakes were estimated using the 113 item semi-quantitative food frequency questionnaire (FFQ) which was utilised in our previous population study of selenium status (15). As a rich source of selenium, participants were also asked about their Brazil nut consumption. Dietary intakes were calculated using Foodworks 2009 dietary analysis software (version 6, Xyris, Brisbane, Australia) and Australian food composition tables (NUTTAB2010) (20). Selenium from supplements and Brazil nuts were included in dietary intakes. Participants also provided a venous blood sample collected into trace element free serum tubes (Becton Dickinson, Rutherford, NJ, USA).

Procedures

Following collection, blood samples were placed on ice and separated within 4 hours by refrigerated centrifugation at 1335 g for 20 minutes. Serum aliquots were stored at -80 °C until analysis. All laboratory glassware and consumables used for trace element analysis were washed with 1% HNO3 before use.

Serum selenium was determined using Zeeman-corrected graphite furnace atomic absorption spectrometry (Spectra 640Z spectrophotometer; Varian, Inc., Palo Alto, CA, USA). Each participant's samples were assayed within the same run to minimise analytical variation. Analysis of Seronorm Trace Elements control sera (Sero, Billingstad, Norway) with certified Se concentrations of 1.36 μmol/l and 2.06 μmol/l gave means of 1.31 μmol/l (CV 5.2%; n = 33) and 2.08 μmol/l (CV 4.8%; n = 13), respectively.

Statistical analysis

The initial subject characteristics of the study population were compared using general linear modelling (GLM) with robust standard error estimation. Associations between serum selenium and selenium intake, regular Brazil nut consumption (at least one serve weekly) and body weight were estimated using repeated measures mixed methods linear regression. Selection of variables for inclusion in a multivariate model was performed using stepwise regression from: selenium intake, regular Brazil nut consumption, body weight, age and gender. P values for entry and removal were 0.12 and 0.20.

The data was grouped by season of collection to determine whether there was any seasonal effect. This seasonal data was compared to spring 2009 (rather than winter 2009 as that time point consisted of the pilot group only). Differences in selenium status between these time points was estimated using repeated measures mixed methods linear regression.

The mean amplitude of variation over 12 months was estimated using repeated measures nonlinear regression using a sine wave model (Y= amplitude*sin ((2*pi*X/wavelength)+phaseshift) adjusted for age and gender.

Post hoc analysis suggested we were able to detect a

variation in dietary intake of 6.5 μg/d and 0.05 μmol/l of serum selenium with a statistical power of 0.8. All analyses were performed using Stata SE12 (StataCorp, College Station, Tx, USA).

Results

Subject characteristics

Of the 91 subjects who participated in the original vitamin D study, 80 consented to participate in this study; of the eleven who did not participate, 9 did not reply to correspondence and 2 were overseas. All subjects attended testing for at least 4 out of 5 time points; numbers at each time point were n = 16 (winter 2009), n = 77 (spring 2009), n = 78 (summer 2010), n = 77 (Autumn 2010), n = 76 (winter 2010) and n = 64 (spring 2010). Reasons for subjects missing time points during the study were illness or interstate/international travel.

At their initial visit, the mean ± SD age of subjects was 69.6 ± 6.5 years , mean BMI was 27.4 kg/m2 and overall dietary macronutrient intake was adequate (Table 1). Only one participant was a current smoker and 29 were taking some form of dietary supplement (only 8 were taking a supplement that contained selenium). Use of supplements, and use of selenium-containing supplements, was not significantly associated with serum selenium (P>0.2).

Men consumed significantly more energy and had a higher absolute intake of selenium (80.6 v. 66.8 μg/d; 95%CI of difference 1.5 to 26.2; P = 0.029), but nutrient density (Se μg/MJ) and selenium intake per kg bodyweight (Se μg/kg) were not significantly different between men and women. While mean selenium intakes were above the Australian estimated average requirement (EAR) of 60 and 50 μg/d (21), one in four men and one in six women had selenium intakes less than the EAR, respectively.

There was no significant gender difference in serum selenium concentrations and the overall mean was below 1.14 μmol/l, a level considered to meet the physiological requirement for the selenoprotein glutathione peroxidase (22); and a commonly used indicator of nutritional adequacy. Almost two thirds (62.5%) of subjects had initial serum selenium concentrations below this level.

Associations with serum selenium

Multivariate analysis (Table 2) showed a significant association between serum selenium and dietary selenium intake (P<0.001) and a negative association with body weight (P=0.008). The greatest effect observed was the regular consumption of Brazil nuts (P<0.001).

Longitudinal analysis

A sine wave nonlinear regression model was used to estimate the amplitude of variation in serum selenium in a seasonal pattern over 12 months. In this analysis the mean serum selenium was 1.11 μmol/l and mean amplitude of variation was 0.02 μmol/l (95% CI -0.7 to 3.6; P = 0.17).

Repeated measures linear regression analysis revealed few significant differences in selenium intakes between time points. The lowest mean intake, observed in winter 2010, was significantly lower than the highest intake

Table 1
Characteristics of study participants at commencement

	All (n = 80)	**Men (n = 23)**	**Women (n = 57)**	
Anthropometric	Mean (SD)	Mean (SD)	Mean (SD)	P
Age, y	69.6 (6.5)	69.5 (5.9)	69.6 (6.8)	0.92
Height, m	1.65 (0.09)	1.74 (0.07)	1.61 (0.06)	<0.001
Weight, kg	74.5 (12.3)	80.8 (10.1)	72.1 (12.3)	0.001
BMI, kg/m^2	27.4 (4.0)	26.7 (3.0)	27.7 (4.4)	0.21
Nutrients				
Energy, MJ	7.89 (2.15)	9.38 (2.24)	7.30 (1.80)	<0.001
Protein, g/d	87.4 (25.3)	103.9 (27.5)	80.8 (21.2)	<0.001
Carbohydrate, g/d	210.7 (58.9)	240.5 (59.9)	198.7 (54.7)	0.003
Fat, g/d	61.5 (19.7)	69.2 (21.2)	58.4 (18.4)	0.030
Dietary Se, μg/d	70.6 (27.2)	80.6 (24.8)	66.8 (27.4)	0.029
μg/MJ	8.81 (2.48)	8.67 (2.18)	8.86 (2.60)	0.74
μg/kg	0.97 (0.41)	1.00 (0.32)	0.96 (0.45)	0.59
Serum Se, μmol/l	1.09 (0.21)	1.11 (0.16)	1.09 (0.23)	0.58

Differences between genders were estimated using GLM

(summer 2010) (Table 3). This difference was relatively small and not reflected in serum selenium. The highest mean serum selenium was observed after the second spring period of the study (spring 2010) compared to the first spring period (spring 2009) and the only autumn period (autumn 2010). As observed in multivariate analysis, regular (at least weekly) consumption of Brazil nuts (a rich source of selenium) was a significant influence on serum selenium and was common in subjects within the upper quartile of serum selenium; 8 (40%) reported regularly consuming Brazil nuts compared to only 3 (5%) from the rest of the cohort. Overall, regular Brazil nut consumers had 30% higher serum selenium compared to non-consumers (1.39 v. 1.07 μmol/l; 95% CI of difference 0.24 to 0.40; P<0.001).

Table 2
Multivariate analysis: the association between mean serum selenium and variables selected by stepwise regression

	Mean	β	95%CI	P
Constant*	1.08		1.04 to 1.12	
Dietary Se, μg/d		0.07	0.04 to 0.11	<0.001
Body weight, kg		-0.05	-0.09 to -0.01	0.008
Regular Brazil nut consumption, yes vs. no		0.24	0.12 to 0.36	<0.001

*Constant is the overall mean serum selenium concentration (μmol/l). The effect of dietary Se intake, body weight (as standardised normal transformations) and regular (at least weekly) consumption of Brazil nuts was estimated using repeated measures mixed methods linear regression. Variables were selected for this model from dietary Se intake, body weight, regular Brazil nut consumption, age and gender using stepwise regression.

Much of the variation observed in the dietary and serum selenium data (Figure 1a and Figure 1b) appeared to occur in those with higher selenium status. For subjects in the upper quartile for serum selenium at their first time point, the mean SD of serum Se change compared to all others was 0.15 vs. 0.07 μmol/l (95% CI of difference 0.04 to 0.13; P<0.001). Subjects who had serum selenium in the upper quartile at their first time point had a 9.9% higher mean serum selenium after 12 months (1.48 v. 1.35 μmol/l; 95% CI of difference 0.04 to 0.23; P = 0.004). For the rest of the cohort the maximum mean difference observed between any two time points was -0.03 μmol/l (95% CI of difference -0.06 to 0.01; P = 0.057).

Figure 1
(a) Dietary selenium intakes and (b) serum selenium concentrations between 2009 and 2010

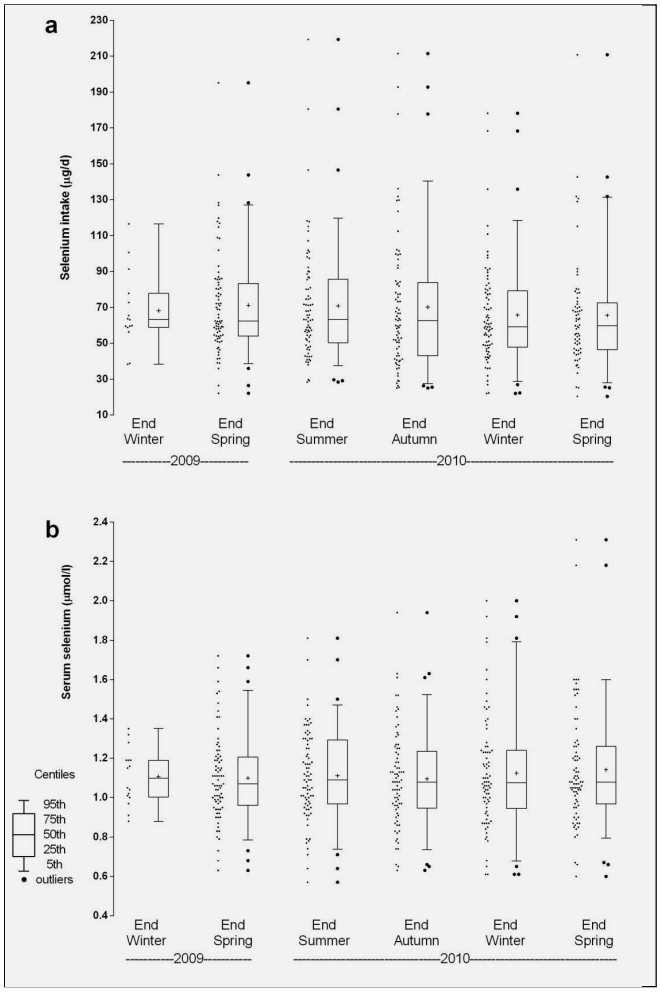

Table 3
Comparisons of dietary and serum selenium for each time point over study period

		Selenium intake (μg/d)			Serum Selenium (μmol/l)		
	N	Mean (SD)	Mean difference (95%CI)	P	Mean (SD)	Mean difference (95%CI)	P
Winter 2009	16	67.8 (19.4)	-2.4 (-11.0 to 6.1)	0.58	1.08 (0.16)	-0.02 (-0.08 to0.05)	0.64
Spring 2009	77	70.3 (29.8)	-	-	1.10 (0.24)	-	-
Summer 2010	78	70.8 (29.9)	0.5 (-4.0 to 5.0)	0.82	1.11 (0.24)	0.01 (-0.02 to 0.05)	0.56
Autumn 2010	77	70.2 (29.8)	-0.1 (-4.6 to 4.5)	0.97	1.10 (0.24)	0.00 (-0.04 to 0.03)	0.91
Winter 2010	76	66.0 (29.7)*	-4.3 (-8.9 to 0.3)	0.07	1.13 (0.24)	0.03 (-0.01 to 0.07)	0.10
Spring 2010	64	67.4 (28.1)	-2.9 (-7.7 to 2.0)	0.25	1.14 (0.23)†	0.04 (0.01 to 0.08)	0.016

Longitudinal variation in selenium status was estimated using repeated measures mixed methods linear regression adjusted for age and gender. Mean differences (95%CI) are for comparisons with spring 2009; * Significantly lower compared to summer 2010 (66.0 v. 70.8 μg/d; 95% CI of difference 0.3 to 9.4; P = 0.038); †Significantly higher than autumn 2010 (1.14 v. 1.10 μmol/l; 95% CI of difference 0.01 to 0.08; P = 0.011).

Discussion

Selenium is important for many aspects of health including immune and antioxidant function. Selenium status decreases with age but is likely to be particularly important in older people who are at increased risk of chronic and other diseases. The aim of this study was therefore to assess selenium status in a cohort of older Tasmanians to determine the magnitude of variation in selenium status over 12 months and investigate the effect of season on dietary intake and serum selenium.

The main finding was minimal variation in selenium status over the 12 month study period. Comparing the individual time points using linear regression, the largest change in dietary intakes was <10%; and <6% in serum selenium. For most of the subjects in this study there was minimal variation in selenium status and little evidence to support the hypothesis that selenium status may vary with a seasonal pattern.

Cross-sectional studies have in the past hinted at possible seasonal differences in selenium status. The British National Diet and Nutrition Survey, assessing selenium status in young people (14), and the elderly (5), reported higher mean plasma selenium concentrations from samples collected in spring months and lower mean concentrations in those collected in autumn months. In our study the highest mean serum selenium occurred in spring; however, the differences between spring and other seasons were very small.

Longitudinal studies of selenium concentrations in human breast milk (23, 24), toenails (25, 26) and serum/plasma (27-29) have been conducted in the past. Most, however, have been long term studies testing for associations between toenail or serum/plasma selenium and various chronic diseases, with measurements of selenium status at intervals of 2 years or longer. There have been few reports to have monitored dietary and serum selenium status over the medium term (up to 12 months) such as the current study; most published data coming from the U.K and Europe. Data from the D-FINES (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England) study (30) (n = 303) was used to estimate selenium intakes in Caucasian and South Asian women (mean age 49 yrs and 50 yrs respectively) over four seasons. A trend of lower selenium intakes in autumn was reported, similar to that from the National Diet and Nutrition Survey (5, 14), in South Asian women but there was no clear evidence of a seasonal pattern. Another British study (31), measured markers of selenium status (including selenium intake, plasma selenium and plasma glutathione peroxidase) in 39 subjects (mean age 45 yrs) at four time points over 23 weeks, and found no effect of time on any markers. In Belgium, Cauwenbergh and colleagues (32) measured serum selenium once per month over one year, in healthy adults (mean age 38.7 yrs). While this study presented ranges of selenium values from the 26 subjects over the 12 monthly time points, the authors did not report comparisons between time points or seasons.

Thus the current study appears to be the first study to assess selenium status repeatedly over 12 months in an older population, and also the first medium-term longitudinal, observational study of dietary and serum selenium status from the southern hemisphere. One of the strengths of this current study, being part of a larger study of vitamin D status, was that subjects were unaware of the focus on selenium and therefore participation in the study was unlikely to have influenced their dietary intake of selenium. The study found little evidence for a seasonal variation in serum selenium status in the cohort overall; this lack of observed variation across the seasons may have been due to older adults tending to eat a less variable diet across the year (33). If there are foods that do exhibit a noticeable seasonal variation in selenium content they simply may not be significant contributors to intakes in this population. In Tasmania, a seasonal pattern has historically been observed (34) in selenium deficiency affecting grazing animals (sheep); the subsequent widespread use of selenium supplementation in grazing animals in this state has potentially removed what may have been the most likely source of significant seasonality in foods for this human population.

The study did again highlight the marginal serum selenium status of this population as described previously (15), with nearly two thirds of subjects having inadequate serum selenium (<1.14 μmol/l) (22). Although men consumed a significantly higher absolute intake of selenium than women, both genders had similarly low serum selenium. This lack of difference has been suggested previously (35) to be due to the greater body mass of men, who had only slightly higher selenium intake per kilogram of body weight than women; this effect was reflected by the negative association between serum selenium and body weight in multivariate regression.

That subjects with relatively low selenium status maintained such a level with little variation over the study period, but those with higher selenium status had a greater variation is particularly interesting. If the lack of seasonal variation in selenium status is reflected in the wider population of older people, it suggests that in the absence of supplementation or other significant dietary change, individuals with marginal selenium status are likely to maintain a relatively consistent selenium status over the medium term, thus avoiding any potential regular declines in antioxidant and immune function associated with decreasing selenium status.

The findings of this study suggest that the inclusion of a single selenium-rich food could have a significant positive effect on selenium status in this older population. Multivariate analysis revealed a strong positive

association between Brazil nut consumption and serum selenium, and consumption of Brazil nuts was common among subjects in the upper quartile of selenium status; observations supporting the reported efficacy of Brazil nuts in increasing selenium status (36). Given the association in the elderly between lower selenium status and impaired glucose metabolism (9), cognitive decline (6) and overall mortality (8), such a dietary inclusion may be worthwhile considering in the future for populations at risk of low selenium status, with the caveat that Brazil nuts can contain varying amounts of toxic metals such as radium and barium (37). Caution may also be required given the potential for adverse effects of increasing selenium status in the selenium replete given recently observed associations of higher serum selenium with diabetes (12) and increased lipid levels (13).

Potential major limitations of the study were the relatively small sample size, recruitment by advertisement (hence potential selection bias) and the methodology used for the dietary analysis. While this FFQ has been used in previous studies (15), the use of a FFQ for dietary assessment has known limitations; and the reliance on food content data that may not be representative of Tasmanian food may have also affected the accuracy of dietary estimates. This might account for the discrepancy between the proportion of subjects with inadequate dietary intake (<EAR) and the proportion with low serum selenium. However, as this study was primarily concerned with the variation between time points, and given the same FFQ was used throughout, the significance of any potential methodological shortcoming is lessened.

In conclusion, selenium status in this cohort of older Australians was marginal but did not vary significantly over 12 months in most subjects and there was no evidence of seasonal pattern.

Funding: This study was supported by a research grant from the Clifford Craig Medical Research Trust (Launceston, TAS, Australia). The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; in the preparation of the manuscript; or in the review or approval of the manuscript.

Acknowledgments: The authors wish to thank Dr Marie-Louise Bird and Jane Pittaway for their planning and management, and data/sample collection during the larger study of which this work was a part. The Royal Hobart Hospital Pathology Department (Hobart, TAS, Australia) assisted with specimen analysis. Dr Iain Robertson (senior medical biostatistician) provided statistical advice.

Conflict of Interest: none declared.

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